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Variations in Apolipoproteins Serum Amyloid A, A-I, A-II, and C-III in Severely Head-Injured Patients

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Summary: In five severely head-injured patients we determined the plasma concentrations of apolipoproteins serum amyloid A, A-I, A-II, C-III, and B, prealbumin and C-reactive protein on day 1, 5, 10 and 15 after head injury where possible. A dramatic increase in apolipoprotein serum amyloid A up to a mean plasma level of 0.764 g/l was accompanied by a considerable decrease in apolipoprotein A-I, apolipoprotein A-II and apolipoprotein C-III concentrations. The variations observed by immunological methods were confirmed by two-dimensional gel electrophoresis performed on plasma and different lipoprotein fractions. In addition to its association with high density lipoproteins, apolipoprotein serum amyloid A was also found with lipoproteins of low and very low density. Two-dimensional electrophoresis also showed the presence of several different serum amyloid A-peptides not seen in plasmas from healthy subjects. We propose that apolipoprotein serum amyloid A may be responsible for the decrease of the main HDL apolipoproteins in head-injured patients.

Introduction

The acute phase response is a systemic reaction to infectious and non-infectious agressive processes. Multiple physiological adaptations occur, including changes in the hepatic synthesis of a number of plasma proteins termed acute phase reactants (1). During inflammation, the apolipoprotein composition of serum high density lipoprotein particles changes markedly. The major apolipoprotein A-I is partially displaced by a protein known either as apolipoprotein S (2–4) or, more commonly, as apolipoprotein serum amyloid A (5–9). Serum amyloid A and C-reactive protein are the most characteristic and sensitive acute phase reactants (10, 11). The synthesis of apolipoprotein serum amyloid A, which occurs predominantly in the liver, increases 500–1000-fold following an inflammatory stimulus (10, 12). Macrophage/monocyte-derived interleukin 1 has been shown to be a potent inducer of serum amyloid A synthesis in hepatocytes (13–16). The mechanism by which interleukin 1 stimulates the synthesis seems to be a direct modulation of the expression of the genes coding for serum amyloid A (17). Recent studies suggest that interleukin 6 and tumour necrosis factor also induce acute phase protein synthesis (18, 19). Up to six isoforms of serum amyloid A have been identified with different isoelectric points ranging between pH 5.2 and 7.4 (20).

Apolipoprotein serum amyloid A is transported in association with high density lipoprotein and may represent as much as 25–50% of the HDL protein (4, 20–22). The effect of the acute response on HDL is of interest since serum amyloid A catabolism rate is affected by binding to lipoproteins (23). *Malmendier* et al. (24) and others (6, 25) have shown that HDL-serum amyloid A seems to be metabilized more rap-

idly than other HDL particles. The particles also containing A-I (Lp A-I: serum amyloid A) have a much slower turnover rate than the particles not associated with apolipoprotein A-I (Lp serum amyloid A) (24).

Several laboratories have recently investigated the properties of HDL from a variety of inflammatory disorders (26–30). All reported a decrease of apolipoprotein A-I concentration. These studies involved comparisons of HDL from normal subjects with HDL isolated at a single time-point from patients with a spectrum of diseases and severity. Effects related to duration and intensity of the acute phase response were not evaluated. The fact that lowered HDL concentrations have been observed in some studies and not in others may be due to differences in the severity of the acute phase responses, or related to the delay in obtaining the plasma after the onset of inflammation. Recently, we have observed an approximate 50% decrease in apolipoproteins A-I, A-II and C-III concentrations after severe head injury (31). The purpose of the present study was to investigate the possible connection of these apolipoprotein changes with apolipoprotein serum amyloid A and its lipoprotein distribution in head-injured patients at different times after the injury.

Materials and Methods

Patients

Five adult patients (3 men, 2 women) were admitted to "Centre d'Orthopédie et de Traumatologie de Strasbourg" for isolated severe head injury. They were apparently healthy before trauma. The patients were daily receiving during their hospitalization a continuous isocaloric enteral infusion of Nutrison® (Nutricia, Holland). This infusion consisted of 36% lipids, 16% proteins and 48% carbohydrates in terms of calories and was supplemented with vitamins and minerals. Of the five patients, two died on day ten after cranial injury, the others survived. Blood samples were collected from each patient on day 1, 5, 10 after injury, also on day 15 from the survivors. Samples were centrifuged and the plasmas were stored at −20 °C until analysis. In these conditions, no significant lipid or apolipoprotein modifications were observed.

Lipoprotein fractionation

Lipoprotein fractions (VLDL, LDL, HDL) were isolated from fresh plasma samples by sequential preparative ultracentrifugation (32) in a Beckman model L5-50 ultracentrifuge, using a Beckman 50 Ti rotor. Fractions were collected and dialysed against a 0.15 mol/l NaCl solution containing Na_2 EDTA (1 mmol/l) pH 7.4 for 24 h at 4 °C. The protein concentration of each fraction was determined as described by *Bradford* (33).

Two-dimensional polyacrylamide gel electrophoresis

Two-dimensional electrophoresis was carried out essentially as described (34) with some modifications for application to apolipoproteins (35, 36). Apolipoproteins were separated in 8 mol/l urea and 20 g/l Nonidet P40 in the first dimension, and 10 g/l SDS in the second dimension. Plasma proteins (200 µg) were applied to the first dimension gel. For VLDL, LDL, HDL analysis, the amount of applied proteins was 6.4, 4.8 and 12.8 µg respectively.

Apolipoprotein determinations

Apolipoprotein serum amyloid A was quantitated using a sandwich ELISA technique (37). Briefly, pure apolipoprotein serum amyloid A was used as primary standard. The antisera were prepared by injecting New Zealand White rabbits with two chemically synthesized short fragments of apolipoprotein serum amyloid A1 corresponding to residues 58–69 and 95–104 of apolipoprotein serum amyloid A covalently linked to tetanus toxoid with glutaraldehyde. The antibodies recognized both their corresponding peptides and apolipoprotein serum amyloid A, and were used in the ELISA assay (37). Apolipoprotein C-III was also quantitated by sandwich ELISA as previously described (38). The apolipoprotein A-I, apolipoprotein A-II and apolipoprotein B concentrations were measured by electroimmunoassay using reagents purchased from Sébia (Issy-les-Moulineaux, France) (39).

CRP and prealbumin deteminations

C-reactive protein concentrations were determined by immunonephelometry as previously described (40). Prealbumin (transthyretin) was assayed with a monospecific antibody (Hyland Division of Travenol Laboratories, Plaisir, France) diluted with a solution containing 40 g of polyethylene glycol 6000 (Merck, Hohenbrunnen, FRG) and 150 mmol of NaCl per litre on a laser PDQ nephelometer Hyland (41).

Results

Five head-injured patients were monitored for 15 days after injury by apolipoprotein measurements on day 1, 5, 10 and 15 (two patients died after day 10, thus no data exist for these subjects for day 15). The results are summarized in table 1. Apolipoprotein A-I and A-II concentrations decreased markedly, while apolipoprotein C-III decreased to a lesser extent and began to rise again on day 15. Whereas apolipoprotein A-I continued to decrease until day 10, the levels of apolipoprotein A-II and C-III had become constant by day 10, or even started to increase again. In contrast, apolipoprotein B concentrations did not change significantly. Apolipoprotein serum amyloid A and C-reactive protein increased immediately after injury. Whereas apolipoprotein serum amyloid A was already increased on the first day and showed a peak on day five, the C-reactive protein increase was retarded, reaching maximal levels on day 10. Prealbumin measured along with the apolipoprotein parameters also decreased in the post injury period.

To further determine whether the increase in plasma levels of apolipoprotein serum amyloid A was due to a preferential increase of one of the plasma isoforms, two-dimensional electrophoresis of plasma was car-

Tab. 1. Plasma concentrations of apolipoproteins (mean ± SD) after head injury. CRP and prealbumin were determined for comparison. Values represent means from five severely head injured patients on day 1, 5, 10 and 15 after injury. Results are expressed in g/l.

Day after head injury	Apolipoprotein serum amyloid A	Apolipoprotein A-I	Apolipoprotein A-II	Apolipoprotein C-III	Apolipoprotein B	C-reactive protein	Prealbumin
1	0.223 ± 0.100	1.39 ± 0.20	0.43 ± 0.15	0.070 ± 0.018	0.68 ± 0.04	0.090 ± 0.090	0.263 ± 0.095
5	0.764 ± 0.260	0.60 ± 0.20	0.04 ± 0.05	0,034 ± 0.019	0.52 ± 0.05	0.262 ± 0.180	0.214 ± 0.046
10	0.555 ± 0.200	0.37 ± 0.10	0.04 ± 0.04	0.044 ± 0.013	0.54 ± 0.12	0.378 ± 0.200	0.149 ± 0.047
15	0.245 ± 0.200	0.94 ± 0.11	0.18 ± 0.11	0.056 ± 0.018	0.63 ± 0.10	0.129 ± 0.036	0.159 ± 0.017
Controls	0.007 ± 0.002	1.40 ± 0.30	0.48 ± 0.18	0.085 ± 0.018	0.85 ± 0.30	0.012 ± 0.010	0.300 ± 0.050

ried out during the study. Figure 1 (a – c) shows the sequence of changes in plasma proteins from a representative patient. In the area which corresponded to apolipoprotein serum amyloid A isoforms according to their physicochemical properties (isoelectric pH, apparent relative molecular mass), two spots were observed at day 1 (fig. 1a). The number and intensity of these spots were increased mainly on day 5 (fig. 1b). At this time six spots were detectable, whose intensity decreased on day 10 (fig. 1c). Figure 1d shows the pattern for a healthy adult; for the same molecular mass, two spots were apparent which might correspond to the two ususal apolipoprotein serum amyloid A isoforms. Thus the increase of apolipoprotein serum amyloid A in plasma was accompanied by the appearance of additional isoforms usually not detected in plasma. On day 5, only two major apolipoprotein serum amyloid A isotypes were detectable in LDL and VLDL. In contrast, the number and intensity of apolipoprotein serum amyloid A spots were more pronounced in HDL (fig. 2). Thus two-dimensional gel electrophoresis gave some information additional to that obtained by the immunological methods.

Discussion

Apolipoprotein serum amyloid A is normally present in trace amounts in human plasma but the concentration increases up to 1000-fold as part of an acute phase response during trauma (27). A tremendous increase in apolipoprotein serum amyloid A has also been observed in the patients studied here with severe head injury.

Several studies have shown that the increase of apolipoprotein serum amyloid A concentration is associated with a low level of apolipoprotein A-I and apolipoprotein A-II or HDL lipids (27, 29). In this study we confirm the "negative acute phase reactant" role of apolipoprotein A-I (30) after head injury and also report a considerable decrease in apolipoprotein A-II and apolipoprotein C-III. Normally apolipoprotein serum amyloid A in plasma is found associated with lipoproteins, especially with those of high density (5). Furthermore, isolated apolipoprotein serum amyloid A polymorphic forms, like other apolipoproteins, have been reported to disrupt multilamellar dimyristoylphosphatidylcholine liposomes and to generate bilayer discs (42). In vitro it has been demonstrated that apolipoprotein A-I can be displaced by apolipoprotein A-II from the HDL surface in such a way that apolipoprotein A-II is transfered from the solvent phase onto the HDL surface in a concentration-dependent fashion (43). Apolipoprotein serum amyloid

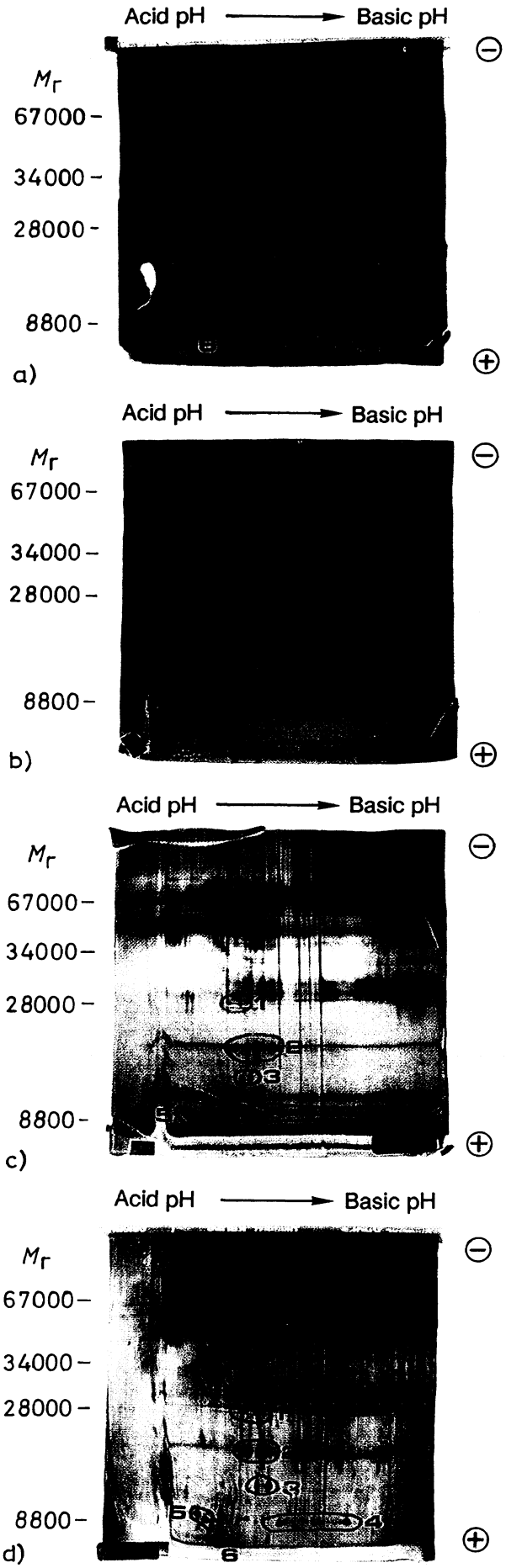

Fig. 1. Two-dimensional polyacrylamide gel electrophoresis of plasma from one head-injured patient on day 1 (a), 5 (b) and 10 (c) after injury in comparison with control plasma (d).
1: apolipoprotein A-I,
2: α_2 chain of haptoglobin,
3: prealbumin,
4: serum amyloid A,
5: apolipoprotein C,
6: apolipoprotein A-II.

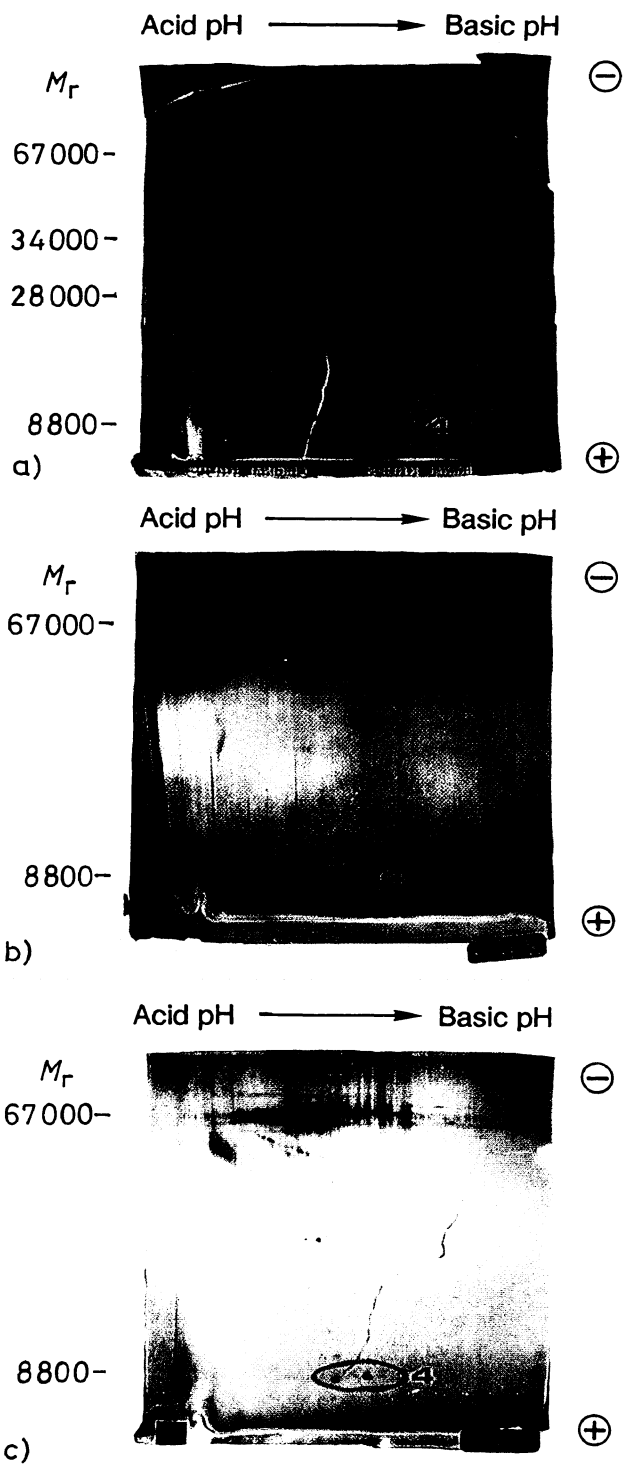

Fig. 2. Two-dimensional polyacrylamide gel electrophoresis of HDL (a), LDL (b), and VLDL (c) isolated from the plasma on day 5 after head injury (same plasma as in figure 1). For details see legend of figure 1.

A also seems to be capable of displacing apolipoprotein A-I or A-II from lipoprotein particles in a very similar, concentration-dependent manner. It was concluded that apolipoprotein serum amyloid A had a higher affinity than apolipoprotein A-I and A-II for HDL particles (29). Several groups have shown that the association of apolipoprotein serum amyloid A mainly with denser HDL_3 particles increased their diameter to form particles that resemble HDL_2 in size (28, 29). The addition of serum amyloid A to HDL resulted in particles comprising up to 80% of serum amyloid A in their protein moiety (29).

One mechanism of lowering of HDL apolipoproteins A-I, A-II and C-III in our study could be their displacement from HDL particles by serum amyloid A. The displaced apolipoproteins would then have an increased catabolism. Several laboratories have postulated that serum amyloid A was incorporated into HDL and displaced the normal HDL protein. This theory is based on two observations. Firstly, serum amyloid A can represent a high proportion of the HDL proteins without a proportional increase in the total protein content (9). Secondly, the incorporation of serum amyloid A into HDL and the concomitant displacement of apolipoprotein A-I have been demonstrated in vitro (29). Serum amyloid A comprised as much as 80% of the HDL protein. Only apolipoprotein A-I was displaced, and the A-II and C protein content did not change (29).

A further mechanism, simultaneously responsible for the decrease of HDL apolipoproteins, might be a reduction of their synthesis during the acute phase response. Hepatic synthesis of several plasma proteins is known to decline during the acute phase response. *Bausserman* et al. (9) suggested that low HDL levels during acute phase response could be due to reduced apolipoprotein synthesis rather than displacement by serum amyloid A. *Baumann* et al. (44) showed a decreased rate of apolipoprotein A-I synthesis in primary hepatocytes isolated after initiation of acute inflammation. Also *Lowell* et al. (45) have reported that the levels of albumin and apolipoprotein A-I mRNA decreased 2-fold in cultured rat hepatocytes after endotoxin administration. Thus several mechanisms, including a direct effect of interleukins 1 and/or 6, may be responsible for the decrease in normal HDL apolipoproteins in head-injured patients (46, 47). Studies of HDL apolipoprotein synthesis by the intact organism and by the liver will be required to demonstrate reduced apolipoprotein production during the acute phase response.

Apolipoprotein serum amyloid A was shown in several studies to be a heterogeneous plasma protein (20, 26, 48–50). Sequencing data from different apolipoprotein serum amyloid A isoforms are compatible with the hypothesis that there exist at least two genes coding for apolipoprotein serum amyloid A (51, 52). cDNA-derived sequences also confirm this view (53, 54). All investigations agree that plasma contains two dominating apolipoprotein serum amyloid A forms (serum amyloid A1 and A2) and up to four minor peptides. Under physiological conditions only apolipoprotein serum amyloid A1 and apolipoprotein serum amyloid A2 are detectable in trace amounts in human plasma. During acute phase responses, not only apolipoprotein serum amyloid A1 and apolipoprotein serum amyloid A2, but also the other isoforms may increase. In our study, head injury led to the multiplication and intensification of certain spots which may correspond to apolipoprotein serum amyloid A isoforms. Present results are compatible with a proportional increase of all isoforms revealing certain spots already present but in indetectable amounts. We also showed that mainly apolipoprotein serum amyloid A1 and apolipoprotein serum amyloid A2 were also associated with LDL and VLDL, whereas the remaining isoforms seem to associate predominantly with HDL. Two dimensional gel electrophoresis during the acute phase response could thus be a tool to monitor the induction of different isoforms in various diseases and to identify possibly amyloidogenic apolipoprotein serum amyloid A forms.

In conclusion, head injury leads to an acute phase response similar to other inflammatory processes; this response includes the appearance of several detectable isoforms of apolipoprotein serum amyloid A in plasma.

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